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Blood-Brain Barrier Permeation in the Rat during Exposure to Low-Power 1.7-GHz Microwave Radiation

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16. ABSTRACT

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Blood-Brain Barrier Permeation in the Rat During Exposure to Low-Power 1.7-GHz Microwave Radiation

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The permeability of the blood-brain barrier to high- and low-molecular-weight compounds has been measured as a function of continuous-wave (CW) and pulsed-microwave radiation. Adult rats, anesthetized with pentobarbital and injected intravenously with a mixture of [^{14}C] sucrose and [^{3}H] inulin, were exposed for 30 min at a specific absorption rate of 0.1 W/kg to 1.7-GHz CW and pulsed (0.5-μs pulse width, 1,000 pps) microwaves. After exposure, the brain was perfused and sectioned into nine regions, and the radioactivity in each region was counted. During identical exposure conditions, temperatures of rats were measured in eight of the brain regions by a thermistor probe that did not perturb the field. No change in uptake of either tracer was found in any of the eight regions as compared with those of sham-exposed animals.

Key words: microwave, blood-brain barrier, 1.7 GHz, pulsed, continuous wave, rat, low-power

INTRODUCTION

The blood-brain barrier (BBB) is a functional concept used to explain selective transport of molecules from the blood into the intracellular compartment of the brain. It also prevents the escape of neuroactive compounds from interstitial fluid into the blood and is probably important in maintaining the internal environment of the brain [Bradbury, 1979]. The barrier has been identified, not as neural tissue, but as the endothelial cells that make up most of the cerebral capillaries [Reese and Karnovsky, 1967]. These cells differ from those of most other tissues by the presence of tight junctions and low pinocytotic activity [Brightman and Reese, 1969; Davson, 1976]. A breakdown of this barrier system must be considered potentially dangerous [Pardridge et al, 1975].

The BBB has been shown to be sensitive to a number of chemical and physical agents [Pardridge et al, 1975]. Within the last several years, several scientists have studied the effects of microwave radiation on the BBB and have reported conflicting results. Some, including Frey et al [1975], Oscar and Hawkins [1977], Chang et al [1982], Albert [1977, 1979], and Albert and Kerns [1981], report increased permeation, while others, eg, Preston et al [1979], Preston and Préfontaine [1980], and

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Gruenau et al [1982], found no changes in permeation. Still others, including Sutton and Carroll [1979], Ward et al [1982], Lin and Lin [1980, 1982], and Merritt et al [1978], found changes, but only in association with large, significant elevations of brain temperature.

Of the reports cited, only those of Oscar and Hawkins [1977] and Frey et al [1975] cite increased permeation at low power levels (< 10 mW/cm^2). Merritt et al [1978] attempted to replicate this work and reported no effects at low power densities. Later Justesen and Baird [1977] reported that Merritt [in a personal communication to Justesen] had reanalyzed his data and found that "his quantitative measure of update of Na-fluorescein was sensitive to microwaves within the range of field strengths employed." Oscar and Hawkins had reported dramatic increases in permeation at relatively low power densities (between 0.03 and 1.0 mW/cm^2). Above 1.0 mW/cm^2, they found that the change in permeation decreased with increasing power (their Fig. 4). This type of curvilinear response led to speculation by Oscar and Hawkins of an "inverted U shaped function." When subsequent literature was reviewed with this in mind, it was noted that at 0.1 mW/cm^2 both Merritt et al [1978] and Preston et al [1979] reported increases in permeation that were not statistically significant. Others, such as Lin and Lin [1980, 1982], Gruenau et al [1982], and Preston and Préfontaine [1980], found no change at similar power levels.

In this study, we used conditions similar to those of Oscar and Hawkins. Similar frequencies, tracers, and exposure durations, and the same modulations (both CW and pulsed) were used in an attempt to reproduce the changes they reported. In addition, an energy-absorption rate was chosen to coincide with that for the maximal changes reported by Oscar and Hawkins. An important difference between this study and that of Oscar and Hawkins is that the latter exposed the whole animal in the far field of an antenna in an anechoic chamber, while we exposed only the head of the animal in a waveguide that operated in the TE_{10} mode.

MATERIALS AND METHODS

Ninety-day-old male CD rats weighing approximately 350 g were purchased from Charles River Animal Laboratories (Portage, MI) and were maintained on water and Purina rat chow ad libitum for at least 1 week prior to treatment.

Blood-Brain Barrier Permeation Measurements

The procedures used were similar to those used previously and have been demonstrated to be sensitive to increased BBB permeation [Ward et al, 1982]. The rats were anesthetized for the duration of the treatments by an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Eight minutes after the injection, both the left and right femoral veins were exposed surgically. Ten minutes after the pentobarbital injection, a mixture of inulin (^{3}H) and sucrose (^{14}C) (New England Nuclear) was injected into the right femoral vein (40 Ci ^{3}H and 20 Ci ^{14}C/kg in a volume of 200 μl/kg). Two minutes after the isotopes were injected, a 0.4-ml blood sample was drawn from the left femoral vein. Five minutes after the injection of the isotopes, the 30-min exposure was initiated. Five minutes after exposure was terminated, a blood sample (0.4 ml) was drawn via cardiac puncture. The chest cavity was opened immediately and the brain was perfused through the left ventricle with 0.9% saline (100 ml). After 10 min of perfusion, the whole brain was removed and nine tissue

samples were prepared by removing the olfactory lobes and then sectioning the brain as described by Glowinski and Iversen [1966] into seven regions: cerebellum, medulla, striatum, hypothalamus, hippocampus, midbrain, and cortex. The cortex was further divided into lateral and frontal sections. The nine samples from each brain were weighed and solubilized in 2 ml NCS (Amersham Searle) at 37 °C for 48 h. When the brain section weighed more than 0.2 g, a 0.2-g portion was removed and used in the subsequent analysis. Then 0.5 ml 3 N HCl and 10 ml of scintillation fluid (ACS, Amersham Searle) were added to each sample and the samples were counted in a liquid-scintillation counter.

The blood samples were allowed to clot and 0.5-ml serum samples were added to 1 ml NCS. After incubation at 37 °C for 48 h, the samples were acidified with 0.25 ml 3 N HCl, and 10 ml of scintillation fluid was added to each. The ^{3}H and ^{14}C activity in the serum samples was measured in a liquid-scintillation counter.

Microwave Exposure System

The microwave exposure system for both the CW and pulsed irradiations is shown in Figure 1. The exposure cell consisted of a 30.5-cm section of WR-430 waveguide with a 3.6-cm-diameter hole drilled in the center of the waveguide's broad wall. Both ends of the waveguide were fitted with waveguide-to-coaxial adapters. The waveguide cell was then placed in a copper-screened wooden box with a hinged acrylic door. A foamed polystyrene platform was placed just below the waveguide access hole for placement of the anesthetized animal. Air temperature was controlled within the screen-lined box by flushing it with temperature-controlled air from a proportional controller (Thermologic Inc., Waltham, MA, Model DIN EA 4211), a blower, and a modified electric space-heater. Air temperature in the vicinity of the subject was monitored by a thermistor probe and temperature readout (Yellow Springs Instruments, Inc., Yellow Springs, OH, Model 46 TUC and a Model 405 probe).

A microwave generator (Eaton Corp., AIL Div., Deer Park, NY, Model 125) operating at 1.7 GHz was used for both the CW and pulsed studies. For the pulsed

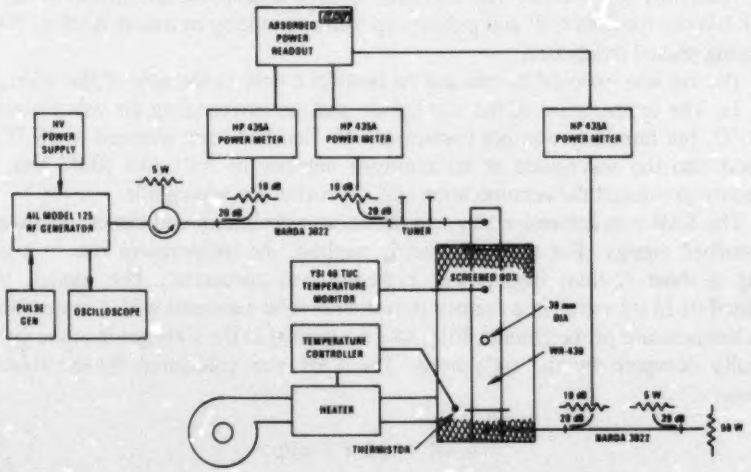

Fig. 1. Diagram of the exposure system for both the CW and pulse RF studies.

RF studies, the generator was modified as recommended in the owner's manual to obtain high peak powers. With this modification, the generator will accept up to 2.5 kV from an external DC power supply. This modification more than doubles the voltage available from the generator's internal supply, thus producing a higher level of peak power. The DC power supply was constructed from components normally intended to service microwave ovens. (Circuit details are available from the authors upon request.) The high-voltage supply provides an adjustable DC output from 0.0 to 2.2 kV at 40 mA. Pulse-repetition rate and pulse width were controlled by a pulse generator that fed the RF generator. Waveshape was monitored at the generator by an oscilloscope.

Microwave energy was routed to the exposure cell through a coaxial circulator, with one port terminated in a 5-W load, then through a bidirectional coupler that was used to sample the incident and reflected levels of power, and lastly through a double-stub tuner that was adjusted for minimal reflected energy while the subject's head was in the waveguide. Microwave energy transmitted through the cell was also sampled and then dumped into a 50-W termination. Three meters (HP Model 435A) were used to measure power levels of forward, reflected, and transmitted energy. The recorder output of each meter was connected to a summing circuit of our own construction, with digital display, which computed the rate of energy absorption by the following equation:

$$P_{abs} = P_{for} - (P_{ref} + P_{trans}) \quad (1)$$

where P_{abs} is the absorption rate, P_{for} is the forward rate, P_{ref} is the reflected rate, and P_{trans} is the transmitted rate.

Exposure Conditions

Both CW and pulsed (1,000 pps, 0.5-μs pulse width) irradiation of the rats was to the head only at 1.7 GHz. The averaged specific absorption rate (SAR) in the head was 0.1 W/kg for both CW and pulsed exposures, resulting in a peak SAR of 200 W/kg during pulsed irradiation.

The rat was exposed by placing its head in a hole in the side of the waveguide (Fig. 1). The temperature of the waveguide and the surrounding air was maintained at 28 °C, but humidity was not controlled. Air that had been warmed to 28 °C was pumped into the waveguide at an averaged velocity of 0.01 m/s (0.07 l/s). This procedure prevented the accumulation of CO_2 within the waveguide.

The SAR was determined by two methods: calorimetry and direct measurement of absorbed energy. For the calorimetric method, the temperature rise in a model during a short (2-min) high-power exposure was measured. The model, which consisted of 15 ml water in a foamed polystyrene tube equipped with a nonperturbing Vitek temperature probe (Model 101), was positioned in the waveguide at the position normally occupied by the rat's head. The SAR was calculated by the following formula:

$$SAR = (\Delta T \cdot c)/t \quad (2)$$

where $\triangle T$ is change in temperature in °C, t is time in seconds, SAR is specific absorption rate in W/kg, and c is the specific heat capacity of water (4,200 J/°C per kg). The $\triangle T$ was measured during the initial linear phase of the heating curve [Allis et al, 1977].

The second method used the summing circuit to measure the rate of energy absorption as described above (equation 1). In this case, the measured rate (P_{abs}) was a combination of absorption by the sample and by the transmission line loss. Line loss was calculated by measuring absorption by a load (P_{abs}) (the rat's head was positioned in the waveguide), then measuring absorption without the load (P_{an}) (the rat's head was removed from the waveguide), with P_{for} and P_{ref} held constant. The rate of energy absorption by the sample (P_s was calculated by $P_s = P_{abs} - P_{an}$; and the ratio P_s/P_{abs} was then determined to be 0.66 for this system. The reading on the summing amplifier (P_{abs}) during exposure of an animal can be corrected by multiplying it by this ratio to yield the rate of energy absorption (P_s). The two methods of measuring the SAR were in good agreement.

Temperature Measurements

A group of 48 animals was used to measure the temperature changes ($\triangle Ts$) in the brain. The $\triangle Ts$ in eight areas of the brain were measured both with and without exposure to microwave radiation. The rats were anesthetized as described above, then the surface of the skull was exposed by splitting the skin and muscle midsagittally on the dorsal surface of the head and gently scraping the muscle aside. The skull was dried with tissue paper, and the site for insertion of the temperature probe was stereotaxically located and marked. A 3 × 3 × 5-mm Plexiglas block with a 1-mm hole was glued (cyanoacrylate ester) to the skull's surface so that the hole was perpendicular to the skull. With the hole in the block as a guide, a 1-mm hole was drilled through the skull. A Vitek probe was next inserted through the block and the skull into the brain to a predetermined depth. Table 1 shows the brain-probe location relative to Bregma (a reference point on the dorsal surface of the skull formed by the intersection of four bones [see Zeman and Innes, 1963]). Initially, the brain was removed and the probe's location was determined by dissection after each experiment. Because the probe's location was never found outside the designated area, this dissection procedure was discontinued midway through the study.

TABLE 1. Brain Probe Coordinates

Region	Anterior (+)/posterior (−)[a] (mm)	Lateral (mm)	Depth (mm)
Cortex	0	2.0	2
Cerebellum	−12.5	1.0	4
Medulla	−11.5	0.5	9
Striatum	+ 2.0	3.0	6
Hypothalamus	− 3.5	1.0	8
Hippocampus	− 3.5	1.0	4
Midbrain	− 3.5	1.0	6
Olfactory lobes	+ 8.8	1.5	4

[a]Anterior/posterior and lateral coordinates are relative to Bregma, while the "depth" coordinate is relative to the surface of the skull. All lateral coordinates were to the left of the midline.

Colonic temperature was measured with a Yellow Springs Instruments (YSI) Model 46 TUC Tele-thermometer and a Model 402 probe, which was inserted 6 cm rectally. There was no microwave interference with the rectal probe because it was not in the field. Although the readout from both of these temperature probes is only to ±0.1 °C, a strip-chart recorder was used to expand the precision of both probes to ±0.05 °C. Ambient air temperature was also measured on a YSI Tele-thermometer and a Model 405 probe that reads to ±0.1 °C.

During these temperature studies, the animal was placed in the exposure chamber in the same manner as described previously. After a 10-min equilibration, the animal either was exposed to microwave radiation (0.1 W/kg; CW only), followed immediately by a sham exposure, or was sham-exposed first then exposed to microwaves. Therefore, half the animals were sham-exposed first and half were microwave-exposed first. All exposures were for 30-min duration at an ambient air temperature of 28 °C.

Analysis of Data

The permeation of the radiolabeled compounds is expressed as a blood-to-brain transfer constant. The transfer constant is the amount of tracer that crosses the capillaries (blood to brain) per unit time; it is an estimate of the product of the brain vascular area and the permeability [Blasburg, 1979].

The transfer constants (K_i) were calculated from the following equation:

$$K_i = T_b \Big/ \int_0^{40} C_a \, dt \tag{3}$$

where T_b is the amount of labeled tracer in the brain region (DPM/g wet mass) at t = 40 min, C_a is the arterial concentration of the labeled compound (DPM/ml), and $\int_0^{40} C_a dt$ is arterial concentration of the tracer from the time of injection until t = 40 min. This integral was determined experimentally by plotting the change of tracer concentration across time and by measuring the area under the curve. An example of the data from an animal injected with ^3H inulin is shown in Figure 2 (solid line). In actual practice the integral was calculated from the 2- and 40-min serum samples (broken line) to obtain an estimated area that was subsequently reduced by a correction factor: the ratio of actual area/estimated area. This ratio for inulin was determined experimentally to be 0.60 (±0.005 SD; n = 3) and for sucrose 0.66 (±0.007 SD; n = 3). This procedure for estimating the integral overcame the technical problems associated with taking repeated blood samples during the 30-min exposures.

RESULTS

Table 2 shows the results from the temperature studies. The change in temperature during sham exposure was compared with the change in temperature during the microwave exposure; the differences were analyzed by the t-test for correlated means. Exposure to microwave radiation is expected to increase but not to decrease the brain temperature; therefore, a one-sided test was used at an alpha limit of P = .10.

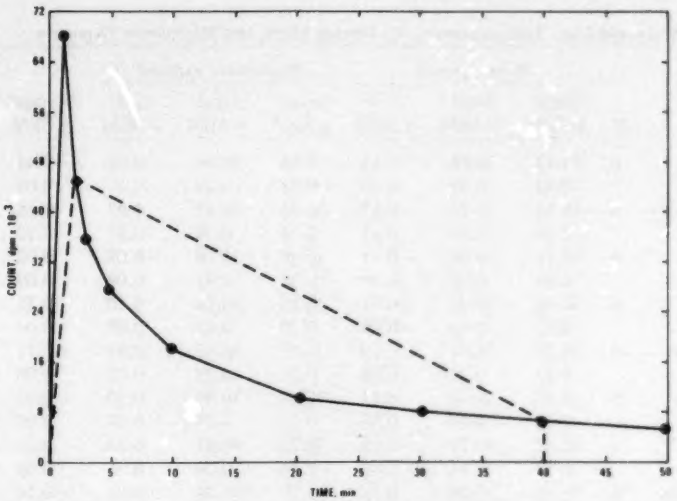

Fig. 2. Comparison of the estimated and true serum level of ^{3}H inulin. The solid line represents the true level of tracer in the serum during the 40-min period, while the broken line shows the estimate of tracer determined by only the 2-min and 40-min serum samples. The ratio of the area under the solid line to the area under the broken line is .60.

Because this analysis was repeated on each brain region for eight analyses, Bonferroni's test for inequality [Lee et al, 1980; Wallenstein et al, 1980] was invoked, and a P value of 0.1/8 (.012) was necessary to reject the null hypothesis. By this criterion, significant $\triangle$Ts were recorded in both the striatum (P = .01) and the midbrain (P = .004) during microwave exposure, with temperature increases of 0.15 and 0.22 °C, respectively. The temperature change for the average of the brain regions was +0.02 °C while the core fell by 0.01 °C compared with controls; neither of these changes is statistically significant. The temperature of the ambient air was very stable over the exposure period with a mean drop of 0.01 °C during the sham period and a rise of 0.03 °C during the exposure period; this difference is not statistically significant.

Mean uptakes of sucrose and inulin for the nine brain regions and the "brain average" are shown in Table 3 and Table 4, respectively. The "brain average" was calculated by first averaging the nine regions for each animal; the brain average was then analyzed in the same manner as that for the individual regions. These data were analyzed by one-way analysis of variance. Microwave exposure, either pulsed or CW, did not result in a significant increase in permeation at the .10 level (even without invoking Bonferroni's inequality) for either sucrose or inulin. The data were also analyzed by Dunnett's test; again no increase in permeation was found.

DISCUSSION

Small temperature increases of 0.15 °C and 0.22 °C were found in the striatum and midbrain, respectively; because of the low averaged SAR (0.1 W/kg), these results were unexpected. If the parameters in this study are used to calculate an expected temperature rise from equation 2 (SAR = 0.1 W/kg; 30 min; heat capacity

TABLE 2. Brain and Core Temperatures (°C) During Sham and Microwave Exposure

		Sham exposed			Microwave exposed				
Region	N	Initial ± SEM	Final ± SEM	Δ T ± SEM	Initial ± SEM	Final ± SEM	Δ T ± SEM	Average[a] ± SEM	P
Cortex	6	34.42	34.58	0.16	34.63	34.64	0.02	−0.14	0.16
		0.42	0.32	0.13	0.32	0.29	0.07	0.09	
Cerebellum	6	36.38	36.26	−0.13	36.43	36.47	0.03	0.16	0.19
		0.36	0.39	0.11	0.34	0.38	0.07	0.10	
Medulla	6	36.81	36.66	−0.15	36.88	36.79	−0.08	0.07	0.47
		0.30	0.25	0.08	0.35	0.32	0.09	0.09	
Striatum	6	36.02	35.98	−0.04	36.13	36.24	0.10	0.15	0.01
		0.51	0.46	0.09	0.50	0.45	0.09	0.04	
Hypothalamus	6	36.23	36.41	0.19	36.37	36.45	0.08	−0.11	0.12
		0.23	0.23	0.08	0.27	0.25	0.12	0.06	
Hippocampus	6	36.37	36.49	0.13	36.44	36.54	0.10	−0.03	0.77
		0.23	0.19	0.06	0.18	0.17	0.07	0.08	
Midbrain	6	36.87	36.79	−0.08	36.73	36.87	0.14	0.22	0.004
		0.13	0.14	0.03	0.16	0.20	0.05	0.04	
Olfactory lobes	6	35.88	36.06	0.18	35.90	35.04	0.04	−0.14	0.04
		0.21	0.20	0.05	0.19	0.18	0.05	0.04	
Brain average	48	36.12	36.15	0.03	36.19	36.24	0.05	0.02	0.48
		0.15	0.13	0.03	0.14	0.14	0.03	0.03	
Core average	48	37.19	37.16	−0.03	37.26	37.23	−0.04	−0.01	0.48
		0.08	0.09	0.03	0.09	0.09	0.04	0.02	
Ambient air	48	28.00	27.99	−0.01	27.95	27.99	0.03	−0.03	0.46
		0.02	0.02	0.02	0.02	0.02	0.02	0.04	

[a]Average is the average difference between the change in temperature during sham exposure and the change in temperature during microwave exposure.

TABLE 3. Brain Uptake of Sucrose*

	Transfer constants ($\times 10^4$, min^{-1})			
Region	Sham, mean ± SEM	CW, mean ± SEM	Pulsed, mean ± SEM	P[a]
Cortex (front)	2.99 ± 0.17	2.99 ± 0.11	2.92 ± 0.10	0.89
Cortex (lateral)	2.78 ± 0.16	2.89 ± 0.12	2.78 ± 0.09	0.79
Cerebellum	2.95 ± 0.17	3.04 ± 0.13	3.00 ± 0.13	0.91
Medulla	2.57 ± 0.14	2.70 ± 0.10	2.65 ± 0.08	0.74
Striatum	2.29 ± 0.19	2.26 ± 0.11	2.36 ± 0.11	0.86
Hypothalamus	2.83 ± 0.20	2.78 ± 0.11	2.81 ± 0.10	0.98
Hippocampus	2.24 ± 0.15	2.23 ± 0.09	2.32 ± 0.11	0.84
Midbrain	2.69 ± 0.14	2.66 ± 0.12	2.73 ± 0.09	0.89
Olfactory lobes	3.48 ± 0.21	3.43 ± 0.16	3.53 ± 0.14	0.91
Average brain	2.76 ± 0.16	2.78 ± 0.09	2.79 ± 0.10	0.98

*n, 9 for each treatment group.
[a]Analyzed by one-way analysis of variance.

TABLE 4. Brain Uptake of Inulin*

Region	Transfer constants ($\times 10^4$, min^{-1}) Sham, mean ± SEM	CW, mean ± SEM	Pulsed, mean ± SEM	P[a]
Cortex (front)	1.87 ± 0.09	2.02 ± 0.09	1.70 ± 0.15	0.14
Cortex (lateral)	1.73 ± 0.09	1.97 ± 0.14	1.62 ± 0.15	0.16
Cerebellum	1.83 ± 0.10	1.93 ± 0.13	1.70 ± 0.13	0.38
Medulla	1.59 ± 0.08	1.65 ± 0.10	1.51 ± 0.10	
Striatum	1.39 ± 0.12	1.49 ± 0.12	1.39 ± 0.16	0.85
Hypothalamus	1.74 ± 0.11	1.76 ± 0.10	1.55 ± 0.13	0.34
Hippocampus	1.47 ± 0.09	1.66 ± 0.13	1.43 ± 0.13	0.39
Midbrain	1.53 ± 0.08	1.54 ± 0.08	1.37 ± 0.12	0.37
Olfactory lobes	1.91 ± 0.14	2.05 ± 0.13	2.04 ± 0.21	0.80
Average brain	1.67 ± 0.09	1.79 ± 0.09	1.59 ± 0.13	0.43

*n, 9 for each treatment group.
[a]Analyzed by one-way analysis of variance.

of brain tissue = 3,700 J/°C per kg), the average ΔT is +0.05 °C (the average ΔT of the temperature measured in brain regions is +0.02 °C). The calculated ΔT yields a heating rate of 2.7×10^{-5} °C/s, assuming uniform energy deposition and no heat loss through blood flow or conduction. With blood flow and conduction dispersing the heat, the temperature gradients within the brain should be small even with nonuniform energy deposition. For these reasons, it was surprising to see statistically significant temperature increases in the midbrain and striatum. The authors, therefore, have reservations about the reproducibility of these findings and believe that these results should be validated before any special significance is attached to them.

Because small but statistically significant increases in temperature were found in two brain regions, the question that should be asked is whether these changes are biologically significant relative to this experiment. The largest difference between sham and exposed results was 0.22 °C over the 30-min exposure period. This difference is also well within normal circadian fluctuations [Isobe et al, 1980]. But more important, a temperature change of this rate and magnitude in itself would not cause a change in barrier permeation [Sutton and Carroll, 1979; Preston, 1982].

This study, although not an exact replication of the work by Oscar and Hawkins, was similar. It was designed to investigate the discrepancies between the work of Oscar and Hawkins and that of Merritt. (Merritt reported "no effects" in his attempt to replicate the changes found in the earlier study.) Table 5 is a comparison of the present study and the Oscar and Hawkins study. The frequencies were close, the pulse parameters were identical, there was overlap in the tracers and energy-absorption rates, and both techniques purported to measure the same endpoint. Three major differences in procedure were the following: 1) different strains of rats were used; 2) exposure conditions—Oscar and Hawkins exposed rats (whole body) in the far field of an anechoic chamber while we exposed ours (head only) in a waveguide; and 3) the present study measured BBB permeation during exposure, while Oscar and Hawkins measured permeation after exposure.

A peak response at a power density between 0.1 and 1.0 mW/cm^2 ($\cong$ 0.02 and 0.2 W/kg) is shown in Figure 4 of Oscar and Hawkins [1977]. Merritt in Table 2 of his manuscript reports almost a twofold increase over control in permeation at a

TABLE 5. Comparison Between Blood-Brain Barrier Studies

Parameter	Oscar and Hawkins[a]	Ward and Ali
Frequency	1.3 GHz	1.7 GHz
Exposure facility	Far field in an anechoic chamber	TE_{10} mode in a waveguide
Exposure	Whole body	Head only
Exposure parameters	Continuous wave and pulsed (1,000 pps, 0.5 μs pulse)[b] $\approx$0.1 W/kg (whole body)	Continuous wave and pulsed (1,000 pps, 0.5 μs pulse) $\approx$0.1 W/kg (head only)
Exposure time	20 min	30 min
Tracers	Mannitol, inulin[b]	Sucrose, inulin
Measuring procedure	Oldendorf technique	Transfer constants
Brain regions	Medulla[b]	Nine regions including the medulla
Strain of rat	Wistar	CD (Sprague-Dawley)

[a]Oscar and Hawkins [1977].
[b]Not inclusive.

power density of 0.1 mW/cm^2, yet because of his method of analysis, he reports "no effects" [Justesen and Baird, 1979]. In this study, just the one dose rate 0.1 W/kg (approximately 0.5 mW/cm^2 [Durney et al, 1978]) was chosen to maximize the statistical power in the event of a nonlinear response.

Some of the pulse parameters (10 μs, 1,000 pps, 2 mW/cm^2 average power density) used by Oscar and Hawkins were duplicated by Merritt. Oscar and Hawkins did find increased permeation in using these pulse parameters, but these conditions were not where the response was greatest. The pulse parameters (0.5 μs, 1,000 pps, 0.1 W/kg) used in the present study were those which were used most often by Oscar and Hawkins and produced some of the largest changes.

The molecular mass of the test compound was shown to be an important parameter by Oscar and Hawkins; increases in permeation were inversely proportional to mass (mannitol > inulin > dextran). Mannitol is a monosaccharide and is roughly half the mass of sucrose, a disaccharide, and consequently should be a more sensitive indicator. Yet Oscar and Hawkins also reported increased permeation with inulin, a polysaccharide, which has 15 times the mass of sucrose. Therefore, although changes in permeation by sucrose may be smaller than mannitol, sucrose should be a sensitive test compound.

Both the Oldendorf technique [Oldendorf, 1970] and the calculation of transfer constants are techniques to measure BBB permeation. We chose to use the calculation of transfer constants because this method is two to three orders of magnitude more sensitive to changes in permeation and it is insensitive to changes in blood flow [Blasburg, 1979].

Despite the similarities in the two studies, we were unable to find increases in permeation reported by Oscar and Hawkins. In a later paper on blood flow, Oscar et al [1981] suggest that "previously reported BBB changes may be less in magnitude than originally indicated." The earlier study [Oscar and Hawkins, 1977] used the Oldendorf technique for measuring BBB permeation, which is sensitive to changes in blood flow; and the later study did find changes in blood flow. Yet a careful comparison of the two reports leads us to a different conclusion; that is, the change in blood flow is not responsible for the reported effects. The only two brain regions

that were monitored in both studies were the cerebellum and the hypothalamus. Increased permeation was found in both regions after a 20-min exposure at 0.5 mW/cm^2 ($\approx$0.1 W/kg). Yet, in the later work, no change in blood flow was found after a 30-min exposure at a higher intensity of 15 mW/cm^2 ($\approx$3.0 W/kg) in either region. This seems to indicate that blood flow was, in fact, not responsible for the observed increase in permeation. The frequencies used in the two studies were different (1.3 GHz and 2.88 GHz), as were the pulse parameters and strain of animal. Perhaps these differences are significant and invalidate a direct comparison. However, if a change in blood flow is responsible for the increased permeation, it is not demonstrated in the later paper. From the results of their paper [Oscar et al, 1981], it does not appear that generalized heating can cause changes in blood flow at low power levels (0.1 W/kg). For a change in blood flow to have been responsible for the effects, the blood flow would have had to change as a result of some more specific interaction such as localized heating or a nonthermal stimulation that may have been frequency dependent.

CONCLUSIONS

In this study we attempted to follow up an earlier study by Oscar and Hawkins [1977] in which pulsed and CW microwave radiation were reported to increase permeation of labeled compounds through the blood-brain barrier. We found no changes in permeation using similar exposure procedures. There were several differences in exposure conditions including strain of rat, tracers, frequency, and microwave-field configuration. Perhaps one of these parameters is responsible for the apparent discrepancy in the results. Yet there is no good evidence to suggest that any of these differences in parameters would lead to such dramatic differences in results.

A review of the literature shows that only Frey et al [1975] and Oscar and Hawkins [1977] report increased permeation at power levels below 10 mW/cm^2. Others who tried to extend this low-level work include Merritt et al [1978], Preston et al [1979], Lin and Lin [1980, 1982], Preston and Préfontaine [1980], Preston [1982], Gruenau et al [1982], and Chang et al [1982] and the present report; none reported any increase in permeation. The Gruenau et al [1982] publication, which was co-authored by Oscar, used a technique by Rapoport et al [1978, 1979], which, like the transfer-constant technique, is insensitive to change in blood flow and is 100 times more sensitive to increased permeation than the Oldendorf technique. Gruenau et al [1982] found no change in permeation. They have "no explanation" other than the possible influence of blood flow for the earlier positive findings of Oscar and Hawkins [1977]. In other words, Oscar seems to be saying that his original effect, if real, is probably a result of increased blood flow. This leaves the report by Frey et al [1975] as the only published manuscript of increased permeation of the BBB below 10 mW/cm^2 in which the author does not attribute the effect to increased blood blow. Frey does not comment on whether he feels that blood flow is responsible for the changes. But as shown in his recent letter [Frey, 1983], he does still feel that the increase in permeation he reported [Frey et al, 1975] is valid and that the data of other scientists (if not their conclusions) also support his view.

Segal and Magin [1983] in a recent review article conclude, "although initial studies suggested that microwaves act to increase the permeability of the BBB, new and improved experiments indicate no specific effect of microwaves aside from those

associated with the concomitant hyperthermia." In light of the complete dominance of "no-effects" findings since the initial reports of low-power (less than 10 mW/cm^2) microwave-induced increased permeation, we must agree with their conclusion—that present evidence indicates that low-level microwave irradiation does not affect the BBB. Yet, we believe it is too early to rule out the possibility of changes in blood flow at these low power levels or that blood flow was responsible for the changes in permeation reported by Frey et al [1975] and Oscar and Hawkins [1977].

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